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Active immunotherapy: current state of the art in vaccine approaches for NHL

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Abstract

Immune therapy of cancer is a rapidly evolving field, with long-deserved successes now finally achieved. As new pathways triggered by the immune synapsis are elucidated, and new molecules responsible for immune checkpoints are being discovered, it is becoming clear that vaccination against a single antigen aided by non-specific immune stimulation is not sufficient for an efficient, long term, immune response. Though lymphoma is a highly curable malignancy, there is still a subset of patients that is at very high risk of disease relapse even after successfully completing chemotherapy or a stem cell transplant. Patients with minimal residual disease are particularly suitable for vaccination. Over the past three decades, the classic model of lymphoma-specific idiotype vaccine has evolved and recent data on vaccination with non specific oligodeoxynucleotides has provided very encouraging results. Furthermore, the introduction of checkpoint blockade via agonist or antagonist monoclonal antibodies holds the promise of significant improvement in the efficacy of future vaccines. What follows is a brief summary of the historical highlights in lymphoma immunotherapy as well as an update on the most recently published clinical trials and a look at future developments.

Keywords

lymphoma; immunotherapy; vaccination; idiotype; adjuvants; checkpoint blockade

Introduction

The mechanism by which the immune system can recognize and kill tumor cells has been explored for more than a century. It is well known that spontaneous tumor regression is not infrequently observed in patients with lymphoma. Unfortunately, it is undeniable that immune surveillance of tumors in their early stages is an imperfect defense mechanism against cancer, and that any significant anti-cancer immunity is offset by the induction of resistance mechanisms, such as down regulation of MHC class I or of tumor associated antigens on the surface of tumor cells (and therefore loss of immunogenicity), the emergence of a subset of T cells or myeloid cells with suppressor function, or the release of tumor-produced immunosuppressive cytokines. Cytotoxic T-lymphocytes (CTLs) that are reactive against tumor antigens can be found in the immune repertoire of cancer-bearing patients. As an example, patients with chronic lymphocytic leukemia (CLL) harbor cytotoxic T lymphocytes that can lyse cells presenting HLA-restricted CD20 or CD19 peptides[1], although these autoreactive CTLs are of low avidity and do not actively kill CLL cells in vivo. Clearly, immune responses to weakly immunogenic antigens are not sufficient for an effective immune rejection of cancer. However, strong anti-tumor immunity can be

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induced by vaccination against tumor associated antigens, immunostimulatory GC-enriched oligonucleotides (CpG) and even self-antigens. While tumor associated antigens are ideal targets because of their uniqueness to the tumor cells, self-antigen have also been shown to be potentially antigenic after specific manipulations, such as epitope optimization[2] or the use of xenogeneic product[3]. In all cases, the induction of humoral and cellular immune responses makes cancer vaccine an attractive alternative to passive immunization.

Lymphoma vaccines: past and present

B lymphocytes express surface immunoglobulins with a specific amino acid sequence in the variable regions of their heavy and light chains, unique to each cell clone (idiotype, Id). When B cells undergo malignant transformation, the idiotype has the potential to function as a true tumor associated antigen. In fact, the clonotypic idiotype of the malignant B cell clone in lymphoma of B cell origin was the first identified tumor associated antigen[4] capable of eliciting a T-cell response[5]. The efficacy of idiotype immunization was initially shown in pre-clinical models of B cell lymphoma. In early clinical work of passive immunotherapy, recombinant murine anti-Id monoclonal antibodies were administered, alone or combined with interferon-α (IFN-α), interleukin-2 (IL-2) or chlorambucil[6]. About half of those patients experienced long-term remissions, longer than those expected with IFN-α, IL-2 or chlorambucil alone. Up to 15 g of recombinant protein/patient were used in those trials, making the cost of such a treatment unreasonable. Moreover, the emergence of Id mutants was a real obstacle to the success of anti-Id therapy[7]. As an alternative, active immunization with a much lower amount of recombinant protein, rather than passive administration of anti-Id antibodies, was used in subsequent clinical trials. Stimulation of immune responses was not elicited when Id alone, a weak immunogen, was administered. The immunoglobulins have to be conjugated to an adjuvant, such as KLH[8], for a meaningful immune response to be elicited. GM-CSF is usually also injected at the site of vaccination as an immune adjuvant. In early studies, the tumor-specific Id was produced by tumor cells-myeloma cell line fusion (hybridoma). Since then, a number of variations on the theme of anti-Id immunizations have been tested, including recombinant DNA technology, in which plasmids producing the tumor-derived immunoglobulins variable regions genes are transfected into bacterial, insect, plant or mammalian cells. Unlike peptide vaccines, plasmid DNA vaccines do not depend on a particular human leukocyte antigen type because the protein product is processed in vivo by host antigen-presenting cells. Intramuscular injection of naked plasmid DNA encoding Id sequences, which results in *in vivo* transfection of muscle cells at the site of injection, seemed, in a small published study, to be less potent at inducing T cell responses than recombinant protein, while none of the patient vaccinated with the Id-producing plasmid mounted an Id-specific humoral response[9]. More potent DNA vaccines are the ones containing single-chain FV sequences derived from the tumor fused to virally-derived immune-stimulatory sequences, designed to generate strong level of T-cell help (and therefore induction of memory B-cells), such as the fragment C of the tetanus toxin-Id fusion[10]. Alternative Id vaccines aiming at easier/faster production or better cost-effectiveness have been explored, such as Fab fragments of the idiotype immunoglobulin produced in E. Coli[11–13].

The advent of immunoinformatics and the availability of software that enable scientists to select the “strongest” epitope for T cell activation based on mathematical models, might possibly further change the design of future vaccines, which might be highly targeted vaccines aimed at specific immunogenic epitopes. Pre-clinical studies of such vaccines, which incorporate selected strong epitopes derived from the tumor cell line's Id and selected tetanus toxoid epitopes were promising, particularly for their easy manufacturing process[14].

Over a period of about two decades, a number of phase I/II clinical trials of idiotype vaccination have been reported (Table 1), most of them in patients with follicular lymphoma. Of note, low tumor burden was thought to be the necessary pre-requisite for the vaccine to induce an efficient response, since constant antigen stimulation can dim an appropriate immune response[15]. Therefore, in almost all the initial studies vaccination began after cytoreduction with a variety of chemotherapy regimens. All these studies demonstrated that Id vaccination was safe. Only minimal side effects were usually reported and they consisted of injection site reactions, such as erythema, induration, swelling and tenderness. The few systemic symptoms observed, such as fever or generalized pain, were thought to be related to concomitant GM-CSF rather than the vaccine itself. *In vitro* antibody and T-cell responses against Id or autologous tumor cells were shown in most studies. Furthermore, clinical efficacy was confirmed by the achievement of complete remissions in patients who had residual disease at the end of induction chemotherapy, or the demonstration of a molecular remission, i.e. the elimination of tumor cells carrying t(14;18) translocation from the blood or marrow, in a proportion of vaccinated patients who had demonstrable minimal residual disease at the end of induction therapy. Overall, the tolerability and efficacy of the Id vaccines in these studies were compelling enough to justify further evaluation in larger, randomized studies.

Pivotal phase III clinical trials of anti-Id immunization

Three randomized clinical trials comparing chemoreduction followed by Id vaccination to chemoreduction alone have been reported[16–18], for a total of nearly 800 patients with follicular lymphoma, either previously untreated or with relapsed disease. The induction therapy was different in all studies and is summarized in Table 2. Notably, Rituximab was part of the induction regimen in two of the trials (Biovest and Favrille), but not in the third (Genitope). It has been shown that previous Rituximab treatment with resulting B cell ablation does not affect the ability of an idiotype vaccine to elicit cellular responses[19]. In all three studies GM-CSF was used as the immune adjuvant, but the Id protein production technique differed among the studies, with the Biovest using hybridoma-produced protein, and the Genitope and Favrille using recombinant protein. In all cases, Id was conjugated to the carrier protein KLH for increased immunogenicity. The results of these studies were, overall, disappointing.

The Genitope trial, which included 287 previously untreated patients, failed to demonstrate a statistically significant improvement in DFS in patients who received Id-KLH plus GM-CSF following induction with CVP chemotherapy compared with patients receiving KLH and GM-CSF without Id. Patients were not required to achieve a response, and patients with stable disease were allowed to receive their custom-made vaccine. Despite the failure to reach the primary endpoint, as expected the proportion of vaccinated patients who indeed mounted an Id-specific immune response had a very significant improvement in PFS compared to the patients who did not. This is an important point, and suggests that the patient's immune milieu at the time of vaccination is central in determining the extent of an effective immune response, and therefore of tumor eradication. Identifying the patients who will benefit from idiotype vaccination might be necessary to move forward with this approach.

The Favrille study comprised 349 patients of which nearly 80% were treatment-naïve. The conditioning regimen consisted of four weekly doses of rituximab. Patients achieving complete and partial responses as well as stable disease after rituximab were allowed to proceed to vaccination. Vaccination was given until disease progression, initially monthly for six doses, then every two months for six more doses and finally every three months until progression. The follicular lymphoma international prognostic index (FLIPI) score was not

balanced between the two treatment arms, with more high risk patients in the experimental arm, possibly confounding the shorter time to first treatment observed in the Id-vaccinated arm compared to the placebo arm. Once the FLIPI score was compounded, the difference between the two arms became no longer significant. Unfortunately, no immune monitoring was performed of these patients' sera and peripheral blood mononuclear cells, and therefore the theory that patients who are able to mount an anti-Id response benefit from Id-vaccination, could not be confirmed in this study.

The most recently reported study, sponsored by the NCI and Biovest, enrolled 234 treatment-naïve follicular lymphoma patients. In the first phase of the study, patients were treated with PACE chemotherapy, but the protocol was later amended to allow for R-CHOP as a more standard induction regimen. Only patients who achieved a CR or CRu following the induction chemotherapy could receive the Id-vaccine. Of note, the vaccine used in this study differed from the one used in the previous two studies in terms of production technique (hybridoma rather than recombinant). Conjugation with KLH and administration of GM-CSF were similar to the other two studies. Two analyses were performed to compare disease-free survival (DFS). The first analysis included all randomized patients (177 of the 234 enrolled). The second analysis included only patients who receive at least one dose of the vaccine (117 of the 177 randomized patients). When all randomized patients (including 60 non-vaccinated patients) were evaluated, no significant difference in median DFS was observed between the groups. However, analysis of the 117 patients who did receive at least one dose of the vaccine revealed a significantly prolonged DFS of 44.2 months versus 30.6 months for the control arm at a median follow-up of nearly five years. Also, patients whose idiotype was of the IgM subtype did extremely well, with a median time to relapse of 52.9 months. Based on these encouraging results, the Biovest vaccine was granted orphan drug status by the FDA. This study was criticized because less than half of the planned number of patients was actually accrued before the study was halted. Also, an important issue to notice in this particular study, is that patients who received the vaccine had to remain disease-free while their vaccine was being produced. The average time to vaccine production was 8 months, so it is possible that the patients who received the vaccine because they remained in remission for that period of time (about two thirds of those assigned to receive it), had less aggressive or less chemoresistant disease. However, it is possible that these data are simply confirming that complete eradication of viable tumor is indispensable to create the most favorable host environment for an appropriate immune response, as it has been previously shown.

All in all, the results of these studies demonstrated a few important points:

- Id-KLH vaccination with recombinant or hybridoma-produced vaccine is safe and well tolerated without long-term side effects
- Patient selection might be necessary based on yet-to-be-defined parameters that might favor the development of a strong immune response
- Achieving a complete remission by effective cytoreductive therapy might be a necessary requisite for this therapy to be successful
- Improved vaccine formulations with the capability of overcoming tolerance, eliciting T-cell help and preventing loss of immunological memory, all being developed, might be the answer to the poor results obtained with the older formulations used in these trials

Beyond idiotype vaccination

While the idiotype represents the model tumor-associated antigen, the three trials described above have clearly demonstrated that the cost and technical difficulties associated with

vaccine production can be limiting factors. In all three trials a vaccine could not be made for a number of patients. Thus, other non-patient specific targets of active immunotherapy for both B-cell and T-cell lymphoma are being investigated, mainly by serological analysis of recombinant cDNA expression libraries (SEREX) technique[20–23]. Cancer-testis antigens in particular, such as MAGE, NY-ESO1 and PASD-1[22,24], are novel, attractive, and relatively specific tumor antigens identified in Hodgkin lymphoma, T-cell lymphoma and diffuse large B-cell lymphoma. Studies of immunization against these antigens are ongoing. Another attractive target for active immunization, given the enormous success of its passive targeting, is the B-cell antigen CD20. In a preclinical model of murine lymphoma, xenogeneic DNA vaccination against CD20 was shown to prevent the development of disease in 20–30% of the animals who received a lethal tumor challenge following CD20 vaccination, compared to unvaccinated mice[25]. In-vivo depletion of CD8+ T cells abrogated the therapeutic effect of the vaccine, indicating a CD8+T cell-mediated mode of action. A phase I, dose-escalation clinical trial in patients with relapsed or refractory CD20+ lymphoma in no immediate need of cytoreductive therapy is ongoing.

Cellular versus protein-based vaccines

As discussed, idiotype vaccination requires the production of custom-made protein for each patient. Another limitation of the strategies described above is that the antitumor response is limited to a single antigen. An alternative to single-antigen protein or DNA-based vaccination is the use of whole tumor cells to elicit immunity against the entire repertoire of antigens expressed by the tumor. Pulsed DC vaccination using apoptotic tumor cells or lysates is a strategy for immunization against tumors that was tested in a variety of preclinical and human studies of lymphoma. Killed tumor cells and tumor cells lysates per se, however, are not sufficiently immunogenic. Dendritic cells (DCs) loaded with tumor cell lysates have been found to elicit impressive antitumor immunity in preclinical models[26] and in small clinical trials[27,28]. Other strategies using DCs to present the full repertoire of tumor antigens expressed by the lymphoma cells include fusion of DCs with tumor cells[29], or pulsing DCs with tumor-derived RNA[24,30,31]. The latter is a promising technique that would require minimal sample size for the amplification of total tumor RNA, thus decreasing the production cost considerably. However, no clinical data is available to date in lymphoma patients.

In situ vaccination

T cell responses can be primed with intratumoral injection of synthetic oligodeoxynucleotides containing unmethylated C-G motifs (CpG). CpG bind to toll-receptor 9 (TLR9) on DCs but also on B cells. Upon binding to TLR9, CpG activate the B-cells antigen presenting machinery that allows them to present tumor-associated antigens to nearby T cells[32]. T cells activated by the interaction of the tumor antigen with the specific tumor-reacting CD8+ T cells secrete IFN-γ and express surface activation markers such as CD137. Vaccination with intratumoral CpG in combination with systemic chemotherapy was initially shown to be effective in preclinical studies of aggressive lymphoma[32]. In a subsequent phase I/II clinical trial, 15 patients with low grade lymphoma received low grade radiation at a single lymph nodal site (to promote release of tumor antigens from irradiated cells) followed by CpG injection at the same site[33]. *In vitro* IFN-γ secretion by autologous T cells after exposure to tumor cells and CD137 expression were used as a measure of effective immune response to CpG vaccination. Assessment of clinical responses at distal sites, including two CRs and two PRs and several ongoing smaller responses, confirmed that a systemic immune response had been created by this approach. The most significant innovation of this “universal” approach to lymphoma vaccination is that no manufacturing of a personalized vaccine is needed, and that the same approach is applicable to B- and T-

cell malignancies. Based on this principle, a similar strategy has been more recently adopted to treat 15 patients with a mature-T cell lymphoma, mycosis fungoides (MF). The fact that a T cell malignancy could be approached with a relatively easy vaccination approach is particularly significant because T-cell receptor-idiotype vaccines and TCR-DNA fusion vaccines, though producing encouraging results in early attempts, are cumbersome to manufacture[34,35]. Of the 15 MF patients treated with the CpG vaccine, 5 had meaningful systemic responses. In the responders, there was a trend towards more pronounced depletion of regulatory T-cells and DCs at the immunized site compared to non-responders, the significance of which is unclear but intriguing.

In a current study for patients with mantle cell lymphoma, based on proof-of-concept pre-clinical studies[36], live tumor cells were co-cultured ex-vivo with CpG and irradiated. This cell-based vaccine was then administered to patients who were previously cytoreduced with standard chemotherapy. The patient's primed T-cells were collected by apheresis, expanded and re-infused together with an extra dose of the vaccine following autologous stem cell transplant. Of twenty-four patients treated as per the last update, seven had measurable CD4+ or CD8+ mediated T-cell responses[37]. Longer follow-up of this study is eagerly awaited.

Checkpoint blockade

The immune system relies on its own inhibitory mechanisms, which serve the purpose of preventing excessive and therefore damaging immune responses. While this controlled-response system is necessary for balanced immunity in normal homeostasis, in the presence of active malignancy the balance is skewed towards excessive inhibition of immune reactivity due to tumor-induced immune suppression and enhanced immunologic tolerance. Lately, several of these immunologic checkpoints have been elucidated. This has resulted in the development of therapeutic immune regulatory antibodies that alter the immunologic checkpoints and consequently enhance immunologic anti-tumor activity. There are two classes of checkpoint antibodies: agonist antibodies that directly enhance the anti-tumor response, and blocking antibodies that inhibit the function of immunologic checkpoint that would otherwise hinder an effective immune response. Anti-CD40, anti-OX40, anti-41BB, anti-CD27 and anti-GITR monoclonal antibodies are examples of agonist antibodies with immune co-stimulatory potential and the ability of boosting anti-tumor T-cell mediated immunity. They have been used primarily in pre-clinical studies as immune adjuvants in various immune therapy settings[38–43].

Anti-programmed death 1 receptor (anti-PD1) and Anti-cytotoxic T-lymphocyte antigen 4 (anti-CTLA-4) monoclonal antibodies down-modulate the negative effect on T-cell activation by the CTLA-4/B7-1/B7-2 and PD1/PD1L checkpoints, respectively. The end result is enhanced signaling through the T-cell receptor. A recent phase III clinical trial of the anti-CTLA-4 monoclonal antibody ipilimumab demonstrated an improved overall survival of patients with metastatic melanoma compared with conventional therapy[44]. Ipilimumab has been tested in patients with lymphoma, with modest but occasionally long-lasting effects[45,46]. Ipilimumab was generally well tolerated. In the Mayo Clinic study[46] two out of seventeen patients achieved an objective response, one being a CR lasting over 31 months at the time of publication.

Similarly, anti-PD1 antibodies block the interaction between PD-1 and its ligand, PD-1L, therefore blocking T-cell activation and proliferation resulting from TCR engagement[47,48]. In a phase I clinical trial of the anti-PD-1 antibody CT-011, clinical benefit was observed in 33% of the patients with one complete remission lasting more than 14 months[49]. A phase II

trial of the combination of CT-011 and rituximab is ongoing[50]. A second, fully humanized, anti-PD-1 antibody, MDX-1106, has only been tested in patients with solid tumors[51].

Combinations of checkpoint antibodies and vaccination have not been tested in humans.

Conclusions

Decades of clinical investigations in cancer vaccines, in hematologic malignancies as well as in solid tumors, have resulted in less than enthusiastic results, lessening the interest that such an innovative approach to cancer therapy had initially produced. However, from years of experience, it is now clear that stimulation of host immunity through vaccination alone is insufficient to elicit an effective anti-tumor immune response and that more targeted modulation of the anti-tumor response (rather than generic stimulation with GM-CSF) is needed. The development of checkpoint antibodies, such as those directed against CTLA-4 and PD-1, and of immune-stimulatory antibodies directed at CD40, 41-BB, GITR, CD27 and OX-40 might eventually change the cancer vaccine landscape and indeed produce more substantial clinical results by shifting the balance of anti-tumor response towards stronger T-cell activation. Combinations of immunomodulatory antibodies and vaccines as well as combination of immunomodulation and epitope-optimized vaccines are needed before closing the chapter on cancer vaccines.

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Table 1

Phase I and II clinical trials of anti-Id vaccination

Author, year	Vaccine	No. of patients	Histology	Immune Responses	Refs.
Kwak, 1992 (updated by Hsu, 1997)	Id-KLH + variable immune adjuvant	41	FL	41 % Ab 17% T	8,52
Bendandi [53], 1999	Id-KLH + GM-CSF	20	FL	75% Ab 95% T	53
Hsu [52, 54], 1996 (updated by Timmerman, 2002)	Id-DC/Id-KLH-DC	35	FL	26% Ab 49% T	27,54
Timmerman, 2002	Plasmid DNA	12	FL	0% Ab 8% T	9
Barrios [55], 2002	Id-KLH + SAF	9	FL	89% Ab	55
Neelapu [56], 2004	Liposomal Id/IL-2	10	FL	40% Ab 100% T	56
Neelapu, 2005	Id-KLH + GM-CSF	26	MCL	30% Ab 87% T	19
Inoges [57], 2006	Id-KLH + GM-CSF	25	FL	52% Ab 72% T	57
Bertinetti [58], 2006	Fab^{Id} + MF59 + GM-CSF	18	Indolent + DLBCL	29% Ab 47% T	58
Redfem [59], 2006	Id-KLH + GM-CSF	31	SLL/CLL, FL	20% Ab 67% T	59
Navarrete, 2011	Fab^{Id}	21	FL, MCL, MZL	50% Ab 55% T	12

Id, Idiotype; KLH, Keyhole Limpet Hemocyanin; GM-CSF, Granulocyte-Macrophage colony Stimulating Factor; DC, Dendritic Cells; FL, Follicular Lymphoma; MCL, Mantle Cell Lymphoma; DLBCL, Diffuse Large B-Cell Lymphoma; MZL, Marginal Zone Lymphoma.

Ab, Antibody response; T, T-cell responses.

Table 2

Phase III Trials of Idiotype Vaccines in Follicular Lymphoma

Author	Sponsor	Idiotype Used	Induction Therapy	Response Required	End Point	Results
Levy et al	Genitope	Recombinant	CVP × 8	CR or PR	PFS	NS
Freedman et al	Favrille	Recombinant	Rituximab × 4	CR, PR, or stable disease	TTP	NS
Schuster et al	NCI/Biovest	Hybridoma	PACE to best response, R-CHOP	CR or CRu	DFS	Significant